Protein-Nucleic Acid Interactions II

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What Structural Elements make a G-Quadruplex Physiologically Viable?

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As much as 375,000 potential G-Quadruplex forming sequences (PQS) have been identified in the human genome nevertheless conclusive demonstration of existence and relevance of these structures *in vivo* has been challenging. Along with the abundance of the PQS, a variety of proteins have also been demonstrated to efficiently destabilize G-Quadruplex (GQ) structures *in vitro*, suggesting the possibility that most GQ forming PQS are rapidly identified and unfolded by these proteins. In an attempt to perform a systematic study of GQ-protein interactions we selected Replication Protein A (RPA) as a model system and studied the interactions of RPA with 10 different GQ constructs with varying loop lengths and number of tetrad layers. RPA is the most abundant single strand DNA binding protein in eukaryotes, is involved in DNA replication and repair along with telomere maintenance, making it an ideal model system for this study. Single molecule FRET and bulk biophysical methods were used to perform these studies. Our results demonstrate a systematic increase in the stability of GQ structures against RPA unfolding as the loop lengths are shortened or the number of tetrad layers is increased. Certain GQ constructs were completely unfolded at RPA concentrations several orders of magnitude less than physiological RPA concentration, suggesting that they would not be viable *in vivo*. Interestingly, the time it takes for an RPA molecule to unfold a GQ construct does not depend on the number of layers the GQ has, suggesting that binding of RPA to GQ is the rate limiting step and that binding affinity decreases as the number of layers is increased. The implications of our data in terms of a molecular understanding of RPA-GQ interactions will be discussed along with our preliminary attempts to model this system.

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Rational Design of Potent Dimeric Ligands as Potential Theraputic Agents for Myotonic Dystrophy Type I (DM1)

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It is known that expanded CUG repeats arising from the DMPK gene sequester the splicing protein MBNL1 and that this leads to myotonic dystrophy type I (DM1). Herein, we describe the rational design and synthesis of a library of dimers that bind with high affinity to CUG repeats. Our goal is to disrupt MBNL1-CUG repeat complex formation. Previously a CUG binding ligand, containing a 1,3,5-triazine-2,4,6-triamine recognition unit linked to an acridine, showed promising MBNL1-(CUG)12 inhibition potency.

The repeating nature of the aberrant RNA, prompted us to follow a bivalent approach through ligand dimerization in order to increase the affinity and selectivity of the ligand. A small library of dimeric ligands was synthesized using linkers with different lengths and functionality. Two different acridine substitution patterns were also examined with the goal of optimizing the bivalent effect. The ability of dimeric ligands to bind to (CUG)12 hairpin and inhibit MBNL1-(CUG)12 complex formation was tested using various biophysical techniques, including Tm measurement, isothermal titration calorimetry, surface plasmon resonance, fluorescence studies and total internal reflection fluorescence microscopy.

The most potent dimer, AJ8, contained N,N′-bis(3-aminopropyl)-1,3-propanediamine, as the linker and was found to inhibit the MBNL1-(CUG)12 complex formation with an IC50 of 1.09 μM in the presence of excess tRNA as a competitor. The inhibition potency is more than 200-fold greater than the corresponding monomer leading to a bivalent effect that is greater than 100-fold. Another advantage of AJ8 is improved drugability profile as it is not cell cytotoxic and unlike the corresponding monomer is aqueous soluble.

This study suggests a general approach by taking advantage of multivalent effect for targeting disorders with repeating causative agents.

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Structural and Functional Consequences of Phosphate-Arsenate Substitutions in Selected Nucleotides: DNA, RNA, and ATP

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A recent finding of a bacterial strain (GFAJ-1) that can rely on arsenic instead of phosphorus raised the questions of if and how arsenate can replace phosphate in biomolecules that are essential to sustain cell life. Apart from questions related to chemical stability, there are those of the structural and functional consequences of phosphate-arsenate substitutions in vital nucleotides in GFAJ1-like cells. In this study we selected three types of molecules (ATP/ADP as energy source and replication regulation; DNA-protein complexes for DNA replication and transcription initiation; and a tRNA-protein complex and ribosome for protein synthesis) to computationally probe if arsenate nucleotides can retain the structural and functional features of phosphate nucleotides. We further compared the experimental EXAFS spectra of the arsenic bacteria with theoretical EXAFS spectra for arsenate DNA and rRNA. Our results demonstrate that while it is possible that dried GFAJ-1 cells contain linear arsenate DNA, the arsenate 70S ribosome does not contribute to the main arsenate depository in the GFAJ-1 cell. Our study indicates that evolution has optimized the inter-relationship between proteins and DNA/RNA, which requires overall changes at the molecular and systems biology levels when replacing phosphate by arsenate.

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Oligomeric Assembly of HIV-1 Rev on the Rev Response Element: Role of Cellular Cofactors

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Rev, a key regulatory protein of HIV-1, activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins, respectively. Initially, a single Rev monomer binds to a highly conserved region, stem IIB located on the Rev Response Element (RRE) of viral mRNA. Following this nucleation step, additional Rev monomers are recruited to the RRE through a combination of RNA-protein and protein-protein interactions, resulting in the formation of a functional nuclear export complex. In addition, several cellular proteins, such as the DEAD box helicases DDX1 and DDX3 are known to be required for efficient Rev function in vivo, although their precise role is unknown. In this study, single-molecule total internal reflection fluorescence (smTIRF) microscopy was used to visualize oligomeric assembly of Rev on the RRE with single monomer resolution. Binding of up to eight fluorescently labeled Rev monomers to a single immobilized RRE molecule was observed in real-time as discrete jumps in fluorescence intensity, and the event frequencies and corresponding binding and dissociation rates for the different Rev-RRE stoichiometries were determined. The smTIRF assay was used to study Rev-RRE assembly in the presence of DDX1, DDX3 and other cellular proteins. The presence of DDX1 promotes oligomeric assembly by accelerating the first few Rev monomer binding steps, suggesting that DDX1 acts as a chaperone of Rev. The smTIRF measurements are being extended to a multi-color format, in order to directly visualize the colocalization of Rev and selected cellular proteins on the same immobilized RRE molecules. These measurements are revealing the precise timing of various protein binding events during ribonucleoprotein assembly. Supported by NIH grant P50 GM082545.

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Histidine-Tag-Specific Optical Probes for Analytical Ultracentrifugation Analysis

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The hexahistidine (His_6)/nickel(II)-nitrilotriacetic acid (Ni^{2+}-NTA) system is a universal tool for the affinity purification of recombinant proteins. Additionally, the NTA group can be exploited for the attachment of fluorophores and chromophores to His_6 proteins at unique user-defined locations. The applications of one such derivative, $(Ni^{2+}\text{-NTA})_2$-Cy3 is characterized. The derivative binds two model His_6 proteins, N-ethylmaleimide sensitive factor (NSF) and O^6-alklyguanine-DNA alkyltransferase (AGT) with moderate affinity ($K \sim 1.5 \times 10^6 M^{-1}$). The activity of these proteins remains unaffected by the attachment of the derivative. The $(Ni^{2+}\text{-NTA})_2$-Cy3 has high specificity which makes it suitable for the detection of His_6 proteins within complex mixtures containing other proteins. This property allows the derivative to be used as a probe of crude cell extracts and as a His_6-specfic gel stain. The binding of the $(Ni^{2+}\text{-NTA})_2$-Cy3 derivative is reversible in the presence of 10 mM ethylenediaminetetraacetic acid (EDTA) or 500 mM imidazole, and in the absence of such agents the exchange occurs slowly ($k_{exchange} \sim 5 \times 10^{-6} S^{-1}$ with 0.2 M labeled protein in the presence of 1 M His6 peptide). The hydrodynamic properties can be explored with the attachment of the $(Ni^{2+}\text{-NTA})_2$-Cy3 derivative utilizing fluorescent anisotropy or analytical ultracentrifugation within environments which prevent direct detection of the protein. Additionally, the $(Ni^{2+}\text{-NTA})_2$-Cy3 derivative can be utilized during fluorescence resonance energy